K053497

Summary of Safety and Effectiveness

APR 14 2006

Submitter Information:

Submitter: Guava Technologies, Inc.
25801 Industrial Blvd.
Hayward, CA 94545

Contact: Leonard Buchner
Director, Quality Assurance
510-576-1374

Name of Device and Classification:

Name: Guava EZCD4 System

Classification: Class II

Predicate Devices:

The Guava EZCD4 Assay is substantially equivalent to the Becton Dickinson MultiTest CD3 FITC / CD8 PE/CD45 PerCP/CD4 APC Reagent (K974360) and TriTest CD3 FITC/ CD4 PE/ CD45 PerCP Reagent (K965053) when performed on the FACSCalibur.

Description of Device:

Determination of the absolute number of $CD4^+$ T cells circulating in the peripheral blood is used to monitor antiretroviral therapy for the treatment of Human Immunodeficiency Virus (HIV) as well as other immunodeficiency diseases. There is an urgent need for an accurate, low-cost, simple, fast, objective and easy-to-learn method for $CD4^+$ T-cell enumeration. Cost containment in the US and Europe has become a primary concern as the reimbursement rates for cytometry-based tests have changed and the need for

more CD4 testing to make good therapy decisions has increased. The Guava® EZCD4™ System was developed to meet those needs.

The Guava EZCD4 System is an optimized cell analysis system consisting of the Guava PCA instrument, EZCD4 software for data acquisition and analysis and an EZCD4 Reagent Kit consisting of optimized reagents and protocols. The Guava EZCD4 Reagent Kit is a two-color direct immunofluorescence kit used for the enumeration of mature $CD4^+$ T lymphocytes in human blood. It consists of a monoclonal anti-human CD3 antibody conjugated to the tandem dye, phycoerythrin (PE)-Cy5 (PECy5) and a monoclonal anti-human CD4 antibody conjugated to PE. Guava 1X Lysing Solution and Guava Fixative are available in separate packaging and are added, after staining, to lyse erythrocytes and to preserve cells respectively.

The Guava PCA instrument includes a laptop computer. The Guava PCA incorporates a new technology termed micro capillary cytometry. The device is a 3-parameter flow cytometer that utilizes a solid state 532 nm green laser, a fixed optical & fluidic system, a self-aligning flow cell and a micro capillary delivery system to perform cell analysis. The micro-syringe stepper pump allows the use of small sample volumes and volumetric measurements for the determination of absolute counts per microliter. The advantages to small sample volumes are low reagent cost and generation of minimal biological waste materials.

The Guava PCA optic system detects forward scatter and two different wavelengths of fluorescence, 580/20 nm and 675/20 nm. It has a small foot print, 21.6 cm x 32 cm x 36.3 cm and is equipped with a network ready laptop computer with a 10GB hard drive and Windows 2000 operating system. The software is application specific with on-screen messaging enabling ease of use and facilitates short, effective training programs. Data is readily analyzed immediately after acquisition and is stored as electronic files on the hard drive.

Description of Device Components

Reagents:

Two antibody reagents and an antibody diluent are included in the EZCD4 reagent kit. The user is responsible for combining these reagents at the time the assay is performed. Anti-human CD4-PE reagent and anti-human CD3-PECy5 reagent are provided in 0.5 mL tubes. The Guava Antibody Dilution Buffer is included in a 1mL tube.

CD4-PE

Clone Edu-2, a monoclonal Mouse anti-human CD4 antibody conjugated to PE. The CD4 antibody allows identification of human helper/inducer CD4+ T Cell (HLA Class II reactive) and recognizes a 60,000 Da MW surface antigen. CD4 is also present on monocytes but at a much lower density and lack co-expression of the CD3 molecule. The CD4-PE monoclonal antibody is purchased directly from Diatec. Incoming QC is performed to ensure antibody meets specification.

CD3-PECy5

Clone UCHT1, a monoclonal mouse anti-human CD3 antibody conjugated to tandem dye PE-Cy5 (PECy5). The CD3 antibody uniquely identifies T-cells and recognizes an epitope on the epsilon chain of the CD3/T Cell antigen receptor (TcR) complex. The CD3-PECy5 monoclonal antibody is purchased directly from Diatec. Incoming QC is performed to ensure antibody meets specification.

Antibody Dilution Buffer

1XPBS, .09% sodium azide and 0.2% BSA pH 7.4.

Guava 1X Lysing Solution

The Guava 1X Lysing solution is a premixed, fixative-free erythrocyte lysing solution. The lysing solution is considered a Class I, General Purpose Reagent (864.4010) and is

produced for Guava by CalTag. The Guava 1X Lysing Solution is sold as a separate reagent.

Guava Fixative

The Guava Fixative is a 20% Paraformaldehye, methanol free solution. Guava orders this component from the original manufacturer and does not perform further processing. The Guava Fixative is sold as a separate reagent.

Guava Check Beads

Guava Check Beads are a suspension of fluorescent imbedded particles at 1×10^6 particles/mL $\pm$ 0.02×10^6 and are a component of the Guava Check Kit. The Guava Check Kit also contains a buffer consisting of isotonic buffered saline solution with 0.2% bovine serum albumin and 0.02% sodium azide as a preservative.

Instrument:

The Guava PCA is composed of a fused silica capillary, a syringe pump to draw fluorescently labeled cells through the capillary, a solid state laser to illuminate the cells as they are drawn through the capillary, detectors to detect scattered light and fluorescent light emitted by the fluorescently labeled cells as they are illuminated by the laser, a waste container into which the sample is pumped after analysis and controls, fluidics and electronics.

Software:

Within the Guava CytoSoft 2.3 version software there are three modules; EZCD4, Guava Check and Clean and Shutdown. Guava Check software controls the instrument for the purpose of determining if the system is functioning properly. EZCD4 is designed to acquire and analyse data specifically for CD4+ T cell results. Clean and Shutdown prepares the instrument for daily shutdown.

Guava Check software controls the acquisition and analysis of data when Guava Check reagent is used. This includes determining when the instrument is ready to start and adjusts instrument settings appropriately for performing a Guava Check run. Once settings are established the software resets the pump and then begins the first of three Guava Check runs. This involves establishing a steady reagent flow, acquiring data (counting events) and then calculating the data received. This cycle is performed three times.

Once the data has been acquired and the instrument is ready for the use, the operator reviews the Guava Check data to ensure it meets pre-determined requirements. If requirements are met, the operator can continue work and begin preparing specimens. If the requirements are not met, the operator must perform system maintenance as required by the operator manual and perform the Guava Check analysis again.

In addition, Guava Check includes a trending features to allow the operator to track instrument performance with Guava Check over time. The operator can track and trend for the last 30, 60 or 90 days. The Guava Check data is stored in a cumulative log.

The EZCD4 module within the CytoSoft version 2.3 software performs many of the same functions as Guava Check software but it is designed for use with actual specimens. The software controls the acquisition and analysis of data for clinical specimens. This includes determining when the instrument is ready to start and adjusts instrument settings appropriately. Once settings are established the software resets the pump and the operator may introduce a specimen for acquisition of data. Sample acquisition involves establishing a steady fluid flow, acquiring data (counting events), analyzing the results and generating a report for review by the medical director for subsequent delivery to the physician.

Clean and Shutdown automatically performs three cleaning cycles and two rinse cycles. Guava recommends running the Clean and Shutdown protocol after each instrument use.

Intended Use / Indications for Use:

The EZCD4 Assay is intended to be performed on a Guava PCA System with CytoSoft 2.3 version software which includes three modules; EZCD4, Guava Check and Clean and Shutdown. The system is intended to identify and quantify the absolute counts of CD4 T-Lymphocytes in EDTA whole blood. The GuavaEZCD4 system is intended for the ongoing monitoring of patients with documented diagnosis of an immunodeficiency disease. The Guava EZCD4 system is intended for use only by trained laboratory professionals.

Performance Data:

Correlation Study

This study was conducted at four clinical trial sites in the US. In each site, the performance of the Guava EZCD4 System was compared with the predicate reagent-instrument system. One of two predicate systems were employed was the Becton Dickinson FACSCalibur™ flow cytometer with MultiTest™ CD3 FITC / CD8 PE / CD45 PerCP / CD4 APC reagents (K974360) or TriTest™ CD3 FITC/ CD4 PE/ CD45 PerCP reagents with TruCount™ Absolute Tubes (K965053). CD4+ T cells enumerated with the Guava EZCD4 (CD3 PECy5$^+$ CD4 PE$^+$) monoclonal antibody was compared with absolute counts enumerated with the Becton Dickinson CD4 (CD3 FITC$^+$ CD4 APC$^+$ or PE+) monoclonal antibody in all sites.

The purpose of this study was to demonstrate that:

1. The Guava EZCD4 System is substantially equivalent to the predicate flow cytometry system for the enumeration of CD4$^+$ T lymphocyte counts.

2. The Guava EZCD4 assay is substantially equivalent to the predicate assay for the detection of CD4$^+$ T lymphocyte counts.

In each study site, approximately 90 whole blood samples were collected from abnormal donors in three CD4$^+$ absolute count ranges. These included approximately 30 donors with counts with the low range (0-200), 30 within mid range (201-500) and 30 within high range (501-2000). The total number of abnormal samples collected and analyzed in the four clinical trial sites was 365.

The linear regression analyses obtained at the four clinical trial sites are summarized on the following table. The regression statistics reported indicate that the results are substantially equivalent.

Linear Regression Analyses

Study Site	N	R squared	Slope	Intercept	Range
2	92	0.95	+ 1.00	18.64	13-1465
3	91	0.93	+ 0.96	35.51	17-1175
4	88	0.98	+ 1.17	18.46	47-1439
5	94	0.98	+ 0.95	13.29	8-1076

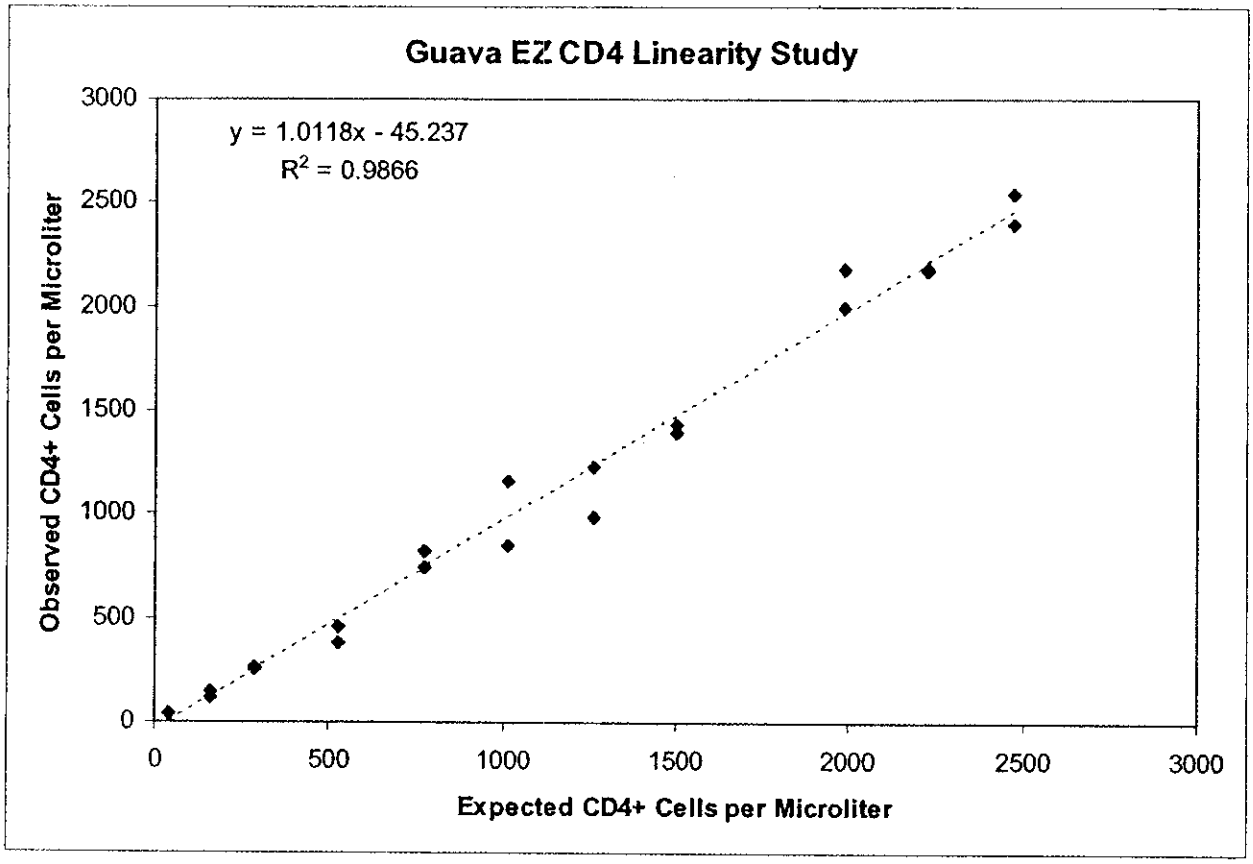
Linearity and Sensitivity Study

This study was designed to demonstrate the linearity of measurement of the Guava EZCD4 System. In this study, *Expected* versus *Observed* values for absolute CD4+ T cell counts were determined by the preparation of a series of blood cell aliquots, each aliquot consisting of a decreasing volume of a bulk blood sample of known "high range" absolute CD4+ T cell counts and an increasing volume of a bulk blood sample of known "low range" absolute CD4+ T cell counts. All cell aliquots were prepared in duplicate and a total of 22 aliquots (11 pairs) were prepared. Testing was performed at Guava Techhologies, Hayward, CA.

The known CD4+ T cell counts of both "high range" and "low range" bulk blood samples combined with the known volumes of bulk blood used to produce the series of blood cell aliquots were used to calculate *Expected* values for absolute CD4+ T cell counts in each aliquot. *Observed* values for absolute CD4+ T cell counts in each aliquot were determined by flow cytometric analysis with the Guava EZCD4 System.

Specific indications were included in the study protocol for the concentration of absolute $CD4^+$ T cells required of the "low range" bulk blood sample (<50 $CD4^+$ T cells/µl) and of the "high range" bulk blood sample (>2000 $CD4^+$ T cells/µl), to also enable the dynamic range of the Guava EZCD4 System to be demonstrated in this study.

The linear regression analysis of the *Expected* versus *Observed* values for absolute CD4 T cell counts with the Guava EZCD4 System are shown in the following graph.



Precision - Intra-Laboratory Reproducibility Study

Intra-laboratory reproducibility of the Guava EZ CD4 System was established by testing at four independent geographically distributed sites in the US. At each site, 10 replicate whole blood samples were prepared and analyzed from each of three abnormal donors representing each of three EZCD4^{+} absolute count ranges. These ranges were Low range (0-200), Mid range (201-500) and High range (501-2000) samples.

Means, standard deviations (SD) and coefficients of variation (CV) were determined for each site in each range and are shown on the following table.

Intra-laboratory Reproducibility

Study Site	Range	Mean EZCD4+ T Cell Counts	SD	CV	N
2	Low	178.41	24.08	13.50	10
	Mid	494.97	39.89	8.06	10
	High	676.45	32.16	4.75	10
3	Low	72.61	7.59	10.45	10
	Mid	417.27	30.06	7.20	10
	High	655.72	32.29	4.92	10
4	Low	81.82	9.04	11.05	10
	Mid	366.36	14.17	3.87	10
	High	870.43	30.99	3.56	10
5	Low	165.44	7.76	4.69	10
	Mid	373.20	13.79	3.69	10
	High	559.65	18.07	3.23	10

Carryover Study

The sample carryover study was designed to determine the presence or absence of sample carryover with the Guava EZCD4 System. The study was conducted in a manner that was most likely to reveal carryover, or the contribution of a previously analyzed sample to a subsequently analyzed sample during sample analysis. This study was conducted at Site 2.

In this study, analysis of three replicates of a low range sample for absolute CD4+ T cell counts (50-200 $CD4^+$ T cells/µL) was immediately followed by the analysis of three replicates of a high range sample for absolute CD4+ T cell counts (500-2000 $CD4^+$ T cells/µL). The procedure of analyzing replicates of the low range sample followed by replicates of the high range sample was continued until seven replicates of the low range sample (n=21) and six replicates the high range sample (n=18) had been analyzed, beginning and ending with replicates of the low range sample.

The following table indicates absolute CD4+ T cell counts obtained for each of the low or high range sample replicates in the Sample Carryover Study.

Carryover Study of Sample Replicates

Sample Numbers	Blood Sample Types	Replicate No. 1 (Absolute CD4 Counts)	Replicate No. 2 (Absolute CD4 Counts)	Replicate No. 3 (Absolute CD4 Counts)
Low 1-3	Low Range Sample	122.67	139.63	133.01
High 1-3	High Range Sample	672.40	765.43	655.53
Low 4-6	Low Range Sample	138.89	113.87	129.49
High 4-6	High Range Sample	684.91	745.29	763.55
Low 7-9	Low Range Sample	144.24	156.11	155.90
High 7-9	High Range Sample	766.90	725.13	784.11
Low 10-12	Low Range Sample	139.64	171.87	146.28
High 10-12	High Range Sample	812.82	732.84	915.32
Low 13-15	Low Range Sample	150.52	150.12	132.84
High 13-15	High Range Sample	734.46	752.94	817.92
Low 16-18	Low Range Sample	138.63	156.83	140.73
High 16-18	High Range Sample	790.88	767.51	805.09
Low 19-21	Low Range Sample	147.68	149.98	136.08

The presence or absence of sample carryover was determined by statistical comparisons of low range samples that immediately precede the analysis of high range samples (Pre-High samples) and with low range samples that immediately follow the analysis of high range samples (Post-High samples). The means, standard deviations (SD) and coefficients of variation (CV) of Pre-High and Post-High low range samples are shown on the following table. The 1 sided p-value of the Wilcoxin-Mann-Whitney test for this comparison was 0.1474.

Statistical Comparison of Pre-High and Post-High Low Range Samples

Low Range Sample Groups	Mean (Absolute CD4 Counts)	SD	CV (%)	n
Pre-High	139.19	9.26	6.65	7
Post-High	143.27	5.04	3.52	6

Based on the data and analyses shown above, the sample carryover study was interpreted to indicate that no significant sample carryover was demonstrated.

Specificity Study

The sample specificity study was designed to determine the specificity of the Guava anti-CD3 (CD3 PECy5) and anti-CD4 (CD4 PE) monoclonal antibody reagents for human peripheral blood lymphocytes. In this study, EDTA anti-coagulated whole blood samples obtained from five healthy subjects were each stained with the Guava anti-CD3 and anti-CD4 monoclonal antibodies and analyzed on the Becton Dickinson FACSCalibur™ flow cytometer. The major white blood cell components, lymphocytes, monocytes and granulocytes from each sample were separately gated and analyzed to determine the percent of antibody positive cells according to the manufacturer's instructions.

The following tables indicate the percent positive cells, means, standard deviations (SD) and ranges for the Guava anti-CD3 and anti-CD4 monoclonal antibodies for each blood component for the five normal donors studied. A total of 15,000 events were collected for the analysis of each blood component for each donor.

Specificity of Guava anti-CD3

Donor Number	Lymphocytes (% Positive)	Monocytes (% Positive)	Granulocytes (% Positive)
1	56.53	5.22	1.14
2	74.04	2.39	1.77
3	23.24	4.53	2.65
4	68.98	2.06	1.63
5	16.20	3.38	0.42
Mean	47.80	3.52	1.52
SD	26.53	1.35	0.82
Range	16.20—74.04	2.06—5.22	0.42—2.65

Specificity of Guava anti-CD4

Donor Number	Lymphocytes (% Positive)	Monocytes (% Positive)	Granulocytes (% Positive)
1	40.09	88.23	4.31
2	43.21	59.92	3.40
3	8.69	80.59	6.06
4	18.04	91.21	6.40
5	7.89	87.94	1.79
Mean	23.58	81.58	4.39
SD	17.00	12.72	1.91
Range	7.89—43.21	59.92—91.21	1.79—6.40

Based on the data and analyses shown above, the sample specificity study was interpreted to indicate the expected specificity of the Guava anti-CD3 monoclonal antibody for peripheral blood lymphocytes. This study was also interpreted to indicate the expected specificity of the Guava anti-CD4 monoclonal antibody for peripheral blood lymphocytes, allowing for the known weak positive staining of CD4 antibodies for peripheral blood monocytes.

Blood Sample Aging

The potential influence of blood sample aging on analysis with the Guava EZCD4 System was evaluated in two separate studies at site 3. In the first study, unstained EDTA anti-coagulated whole blood specimens were stored for defined periods of time and then stained and analyzed with the Guava EZCD4 System (Pre-Staining Study). In the second study, EDTA anti-coagulated whole blood samples were first stained, lysed and fixed, then stored for defined periods of time and temperature and then analyzed with the Guava EZCD4 System (Post-Staining Study).

Pre-Staining Study

In this study, five EDTA anti-coagulated whole blood specimens were drawn from each of five healthy normal donors. Each of the five unstained blood specimens was stored at 18-25°C for 0, 8, 24, 48 and 72 hours. Each of the five blood specimens was

immediately stained and analyzed with the Guava EZCD4 System for the determination of absolute CD4+ T cell counts at the end of their respective storage periods.

The absolute CD4+ T cell counts were determined for each blood sample from each donor at each storage interval. Means, standard deviations (SD), coefficients of variation (% CV) and percent change over time were determined for each donor blood sample. These values are shown in the table ("Pre-Staining Study") on a following page. Also shown is a plot of the percent difference of samples evaluated over the indicated time intervals.

Post-Staining Study

In this study, five EDTA anti-coagulated whole blood specimens were drawn from each of five healthy normal donors. Seven samples were stained for each of the five blood samples drawn, and were then pooled and stored at 18-25°C for 0, 4, 8 and 24 hours. Each of the five pooled samples was analyzed with the Guava EZCD4 System for the determination of absolute CD4+ T cell counts at the end of their respective storage periods.

In this study, five additional EDTA anti-coagulated whole blood specimens were drawn from each of five healthy normal donors. Seven samples were stained for each of the five blood samples drawn, and were then pooled and stored at 2-8°C for 0, 4, 8 and 24 hours. Each of the five pooled samples was analyzed with the Guava EZCD4 System for the determination of absolute CD4+ T cell counts at the end of their respective storage periods. In order to facilitate 0 hour testing, the same 0 hour blood samples were analyzed in the 2-8°C and 18-25°C portions of this study.

The absolute CD4 T-cell counts were determined for each blood sample from each donor at each storage interval. Means, standard deviations (SD), coefficients of variation (% CV) and percent change over time were determined for each donor blood sample. These values are shown in the table ("Post-Staining Study") on a following

page. Also shown is a plot of the percent difference of samples evaluated over the indicated time intervals.

Based on the data and analyses shown, the Pre-Staining Study was interpreted to indicate that unstained EDTA anti-coagulated whole blood specimens may be stored for periods up to and including 72 hours, prior to staining and analysis with the Guava EZCD4 System.

Based on the data and analyses shown, the Post-Staining Study was interpreted to indicate that stained EDTA anti-coagulated whole blood specimens may be stored for periods up to and including 24 hours, prior to analysis with the Guava EZCD4 System.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Guava Technologies, Inc.
c/o Mr. Lawrence F. Bruder
President and CEO
25801 Industrial Blvd.
Hayward, CA 94545

APR 14 2006

Re: k053497
Trade/Device Name: Guava EZCD4 System
Regulation Number: 21 CFR 864.5220
Regulation Name: Automated differential cell counter
Regulatory Class: Class II
Product Code: GKZ
Dated: December 15, 2005
Received: December 15, 2005

Dear Mr. Bruder:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

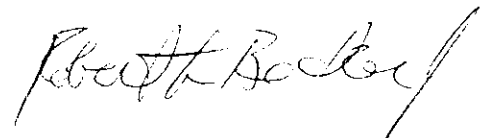
If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of Compliance at (240) 276-0484. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,



Robert L. Becker, Jr., M.D., Ph.D.
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use Statement

510(k) Number: K053497

Device Name: Guava EZCD4 System

Indications For Use:

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Prescription Use __X__ OR Over-The-Counter Use _____
(per 21 CFR 801.109)

(Optional Format 1-2-96)

Maria Chan for Josephine Bunte

Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) K053497